

Utility Patent
033134.2097.UTL1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

John Reidhaar-Olson**Serial No.:** 09/489,220**Filed:** January 21, 2000**For:** TOXICANT-INDUCED
DIFFERENTIAL GENE EXPRESSION

Examiner: Frank Lu

Art Unit: 1655

AMENDMENT AND RESPONSE TO OFFICE ACTIONCommissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Office Action mailed January 29, 2002 ("Paper No. 16"), Applicants respectfully request that the Examiner enter the following amendments and consider the following remarks.

CERTIFICATE OF TRANSMISSION
(37 C.F.R. §1.8)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being facsimile transmitted to the United States Patent and Trademark Office on 4/29/02.

Signature

Wendy I. Therrien

Name of Person Signing Certificate

Wendy I. Therrien17/D
5/13/02
CD

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IN THE CLAIMS

Please amend claim 1 as follows:

1. A method for detecting a toxic response, comprising:

- (a) contacting test cells with a compound;
- (b) determining the expression levels of two or more nucleic acids in a test sample,

wherein the two or more nucleic acids are selected from the group consisting of Putative cyclin G1 interacting protein, EST (W74293), Fatty-acid -coenzyme A ligase (long-chain 3), KIAA0220, KIAA0069, Acinus, Translation initiation factor eIF1 (A12/SUI1), Ornithine aminotransferase (gyrate atrophy), Insulin-like growth factor binding protein 1, Metallothionein-1H, F₁F₀-ATPase synthase *f* subunit, Ring finger protein 5, EST (H73484), XP-C repair complementing protein, Squalene epoxidase, Microsomal glutathione-S-transferase 1, Defender against cell death 1, COPII protein, KIAA0917, Corticosteroid binding globulin, Calumenin, Ubiquinol-cytochrome c reductase core protein II, SEC13 (*S. cerevisiae*)-like 1, EST (R51835), Human chromosome 3p21.1 gene sequence, EST (AA 441895), Ribonuclease (Rnase A family, 4), Transcription factor Dp-1, MAC30, Cyclin-dependent kinase 4, Multispanning membrane protein, Splicing factor (arginine/serine-rich 1), Cytochrome c-1, Lactate dehydrogenase-A, Pyrroline-5-carboxylate synthetase, Glutamate dehydrogenase, Pyruvate dehydrogenase (lipoamide) beta, Ribosomal protein S6 kinase (90kD, polypeptide 3), Acetyl-coenzyme A acetyltransferase 2, Proteasome activator subunit 3 (PA28 gamma; K_i), EST (N22016), EST (AI131502), Activating transcription factor 4, Transforming growth factor-beta type III receptor, Glutathione-S-transferase-like, NADH dehydrogenase subunit 2, Heat shock protein 90, the cAMP-dependent transcription factor ATF-4, EST(AI148382), EST (AA283846), EST

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(AI310515) and EST (AA805555), wherein the numbers listed in parentheses is the GenBank accession number; and

D1 (b) comparing the expression levels in the test sample with expression levels of the same nucleic acids in a control sample, wherein a difference in expression levels between the test and control samples is an indicator of a toxic response in the test sample.

Please amend claim 7 as follows:

D2 7. The method of claim 1, wherein the group consists of Cytochrome c-1, F₁F₀-ATPase synthase f subunit, Ubiquinol-cytochrome c reductase core protein II, Lactate dehydrogenase-A, Pyruvate dehydrogenase E1-beta subunit and NADH dehydrogenase subunit 2.

Please amend claim 9 as follows:

D3 9. The method of claim 1, wherein the group consists of XP-C repair complementing protein, Microsomal glutathione-S-transferase 1, Glutathione-S-transferase-like, Metallothionein-1H, Heat shock protein 90, cAMP-dependent transcription factor ATF-4 and EST (AI148382).

INFORMATION DISCLOSURE STATEMENT

Attached is a photocopy of the postcard received from the Patent and Trademark Office acknowledging receipt of the Information Disclosure Statement submitted in connection with this application. Please verify that this Information Disclosure Statement and the accompanying

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references cannot be found and advise if Applicant needs to re-submit the Information

Disclosure Statement.

REMARKS

The present invention concerns the measuring the differential expression of a selected set of genes in response to exposure to toxicants. Applicant has selected a set of genes whose change in expression is indicative of a response to a toxic substance. By restricting the assay to this select group of genes, Applicant has reduced the time and expense required for such an assay. Rather than, for example, buying gene chips to assay for all possible human genes, Applicant has set forth a list of genes which could easily be contained on a single gene chip.

The claims have been amended to more clearly describe the claimed invention. Support for these changes may be found as follows, *inter alia*:

- (1) NADH dehydrogenase subunit 2 is listed on page 4 at line 26, and in Appendix A on page 111;
- (2) Heat shock protein 90 is listed on page 4 at line 21, and in Appendix A on pages 104 and 112;
- (3) The cAMP-dependent transcription factor ATF-4 is listed on page 3 at line 1, on page 4 at lines 21-22, and in Appendix A on page 111;
- (4) EST(AI148382) is listed on page 4 at line 22. Comparison of the GenBank entries for AI148382 and AI131502 reveals that they are identical except that AI13102 has an additional two thymidines at the 3' end of the clone. AI13102 is listed on page 3 at line 2 and in Table 1 on page 33;

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- (5) F_1F_0 -ATPase synthase *f* subunit is listed on page 2 at line 20, in Table 10 on page 100, and in Table 12 on page 102. Further, it is a subunit of the F_1F_0 -ATPase synthase listed on page 4 at line 24;
- (6) Glutathione-S-transferase-like is listed on page 2 at line 26, in Table 1 on page 33, in Table 10 on page 100, and in Table 12 on page 102. Microsomal glutathione-S-transferase 1 is listed on page 2 at line 22, in Table 1 on page 33, in Table 10 on page 100, and in Table 12 on page 102; and
- (7) Contacting test cells with a compound is described at page 17, line 32 to page 18, line 12. Page 2 explains that a subset of the list of differentially expressed genes of the instant invention are selected for an assay of the instant invention. The full set of differentially expressed genes which may be selected are listed in Appendix A, while a selected subset of these genes is listed in Table I. For example, see page 4, line 17, *et seq.* However, any of the genes listed in Appendix A may be selected for the assay of the instant invention.

The amendments to the claims are fully supported by the application as filed, add no new matter, and should not be construed as limiting the appropriate scope of protection under the doctrine of equivalents.

A. Claim Objections under 37 C.F.R. 1.75(c)

The Examiner has objected to claims 7 and 9 as being allegedly improper with regards to the dependent form for failing to further limit the subject matter of the previous claim. Applicant respectfully submits that the objection no longer applies to the claims as amended and that the Examiner withdraw this objection.

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B. Claim Rejections under 35 U.S.C. Section 112

Claims 1-19 (claims 2 through 19 are dependent on 1) are rejected as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claim 1 is rejected as allegedly being incomplete for omitting essential steps where the Examiner alleged that the "omitted step is to incubate a test sample with a toxic material." Applicant respectfully submits that the objection no longer applies to the claim as amended and respectfully requests withdrawal of the objection.

The Examiner has objected to the limitation in claim 7 of the F_1F_0 -ATPase synthase and NADH dehydrogenase subunit 2 due to an alleged insufficient antecedent basis. Applicant has amended claim 7 to specify the f subunit of the F_1F_0 -ATPase synthase. Support for this modification is found at page 2, line 20. Applicant respectfully submits that the specification identifies differentially expressed nucleic acids grouped by the present invention in Appendix A (page 2, lines 10-11). NADH dehydrogenase subunit 2 is listed in Appendix A on page 111, and has been added to claim 1. Applicant respectfully submits that the objection no longer applies to the claim as amended and respectfully requests withdrawal of the objection.

The Examiner has objected to the limitation in claim 9 of the Glutathione-S-transferase, Heat shock protein 90, cAMP-dependent transcription factor ATF-4 and EST (A1148382) as lacking insufficient antecedent basis. The terminology of claim 9 has been adjusted to match the antecedents in claim 1 with regard to ATF-4 and Glutathione-S-transferase-like. With regards to Heat shock protein 90, Applicant respectfully submits that the specification identifies differentially expressed nucleic acids grouped by the present invention in Appendix A (page 2, lines 10-11). Heat shock protein 90 is listed in Appendix A on pages 104 and 112, and has been

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added to claim 1. Further, comparison of the GenBank entries for AI148382 and AI131502 reveals that they are identical except that AI13102 has an additional two thymidines at the 3' end of the clone. (See below.) AI13102 is listed on page 3 at line 2 and in Table 1 on page 33. Applicant respectfully submits that the objection no longer applies to the claim as amended and respectfully requests withdrawal of the objection.

Sequences of AI14832 and AI13102 from GenBank:

AI148382. qcl4e09.xl Soares...[gi:3676851]
IDENTIFIERS

dbEST Id: 1907084
EST name: qcl4e09.xl
GenBank Acc: AI148382
GenBank gi: 3676851

CLONE INFO

Clone Id: IMAGE:1709608 (3')
Source: NCI
Insert length: 2287
DNA type: cDNA

PRIMERS

Sequencing: -40m13 fwd. ET from Amersham
PolyA Tail: Unknown

SEQUENCE

TTTTTTTTTTTTTTTGTAAATCAAATTTACTTTTATTCACAAATTATTTTTTCAAACAT
TTACTACATTGAAATAAAAAATTTATCAACAAAATATTTAAATCTGGTTTATAATTTTGAT
TTTTAAAGTGAGGAAAATTCTACCTTGGCAGTGAAGACAGCCTGTCTTGCCTCAGGTATC
ATATAAAGTTGCTGAATAGTAGAAGCTAAGTAACAAGTAGCTCCAAGGTTAGTAAGGCCA
ACAAATCTACATTACGACGACATCTTCATGAGGCCAGTAATCCCATTATAAGGTGCA
TGGGACTGCATGTGTGTGCCATAACCCAGTTGTGTATTAGCCTGTAGTTCTCAACAGAC
CCCTTTACCATCTCTACTAACAATCGTAAGCGGCAGCTCTTGAAGAATGTGATTGAC
TTTGGCTGTT

AI131502. qc13f07.xl Soares...[gi:3601518]
IDENTIFIERS

dbEST Id: 1890024
EST name: qc13f07.xl
GenBank Acc: AI131502
GenBank gi: 3601518

CLONE INFO

Clone Id: IMAGE:1709509 (3')
Source: NCI
Insert length: 1259
DNA type: cDNA

PRIMERS

Sequencing: -40m13 fwd. ET from Amersham
PolyA Tail: Unknown

SEQUENCE

TTTTTTTTTTTTTTTGTAAATCAAATTTACTTTTATTCACAAATTATTTTTTCAAACAT
TTACTACATTGAAATAAAAAATTTATCAACAAAATATTTAAATCTGGTTTATAATTTTGAT
TTTTAAAGTGAGGAAAATTCTACCTTGGCAGTGAAGACAGCCTGTCTTGCCTCAGGTATC
ATATAAAGTTGCTGAATAGTAGAAGCTAAGTAACAAGTAGCTCCAAGGTTAGTAAGGCCA
ACAAATCTACATTACGACGACATCTTCATGAGGCCAGTAATCCCATTATAAGGTGCA

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TGGGACTGCATGTGTTGTGCCATAACCCAGTTGTGTATTAGCCTGTAGTTCTCAACAGAC
CCCTTTACCATCTCTACTAACAAATCGTAAGCGGCAGCTCTTGAAGAATGTGATTGACAC
TTTGGCTG

C. Claim Rejections under 35 U.S.C. Section 102

The Examiner has rejected claims 1, 7, 11, 14, 18 and 19 under § 102(b) as being anticipated by Luciakova, *et al.* (1992). Applicant respectfully submits that the F_1F_0 -ATPase synthase beta-subunit is not listed in claim 1. Further, Applicant respectfully disagrees that serum deprivation is encompassed by the invention. The present invention clearly involves the step, *inter alia*, that test cells are contacted with a toxicant, not deprived of an item essential for growth. Applicant respectfully disagrees that serum deprivation or growth activation could be considered a toxic insult. Therefore, Applicant respectfully requests that the Examiner withdraw the objection.

The Examiner has rejected claims 1, 9, 11, 14, 18 and 19 under § 102(b) as being anticipated by Nemoto, *et al.* (1995). Applicant respectfully submits that the glutathione-S-transferase (GST) referred to by Nemoto *et al.* is obtained from a parasite, *Schistosoma japonicum*, and has been incorporated into the pGEX vectors offered for sale by Pharmacia (See the Pharmacia web site at www.apbiotech.com/na/ for a description of the pGEX vectors.). Following the instant amendments, this glutathione-S-transferase gene not referenced in claims 1, 9, 11, 14, 18 and 19. Applicant respectfully requests that the Examiner withdraw the objection.

D. Claim Rejections under 35 U.S.C. Section 103

The Examiner has rejected claims 1-11, 14, 15, 18 and 19 under § 103(a) as being unpatentable over Diel, *et al.* (1995) in view of Fagan (1989). Applicant does not dispute that

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Diel, *et al.* measured the response of gene expression in liver cells to estrogen, and further, that one of the genes studied by Diel, *et al.* was IGFBP-1. Diel *et al.* were identifying liver genes that demonstrated an increased level of expression in response to estrogen. Diel, *et al.* identified candidate genes using ddRT-PCR, then confirmed results with Northern blots. Diel *et al.* first identified ten bands on a ddRT-PCR gel, and correlated published sequences with three of them after sequencing. Additionally, Diel *et al.* suggest the use of IGFBP-1 as having the "potential of being applied as a sensitive marker for hepatic estrogen action *in vitro* and *in vivo*." In this study, Diel *et al.* were attempting to discern which genes might be regulated in response to a specific endogenously-produced hormone. They were not attempting to assemble a group of genes which can be used to compare the effects of disparate, potentially exogenous, chemicals.

Applicant further agrees that Fagan *et al.* describe the level of OAT mRNA and protein in retinoblastoma cell lines. Further, Fagan *et al.* report that OAT mRNA expression is increased in these cell lines following exposure to estrogen. Fagan *et al.* also report the study of Mueckler *et al.* (J. Biol. Chem. 259:2302-05, 1984) which showed that estrogen did not stimulate OAT transcription in rat liver. Since one skilled in the art interested in measuring the response of mRNA levels to a toxicant would likely be particularly interested in such a response in the liver, such information would be seen as teaching away from selecting the OAT gene to measure toxic response.

Applicant wishes to point out that the literature of the time was replete with studies where cells were stimulated with a variety of chemicals in order to measure the changes in the level of gene expression. Applicant respectfully argues that the Examiner has failed to provide any motivation for one skilled in the art to combine the two references and simultaneously measure

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the response of IGFBP-1 and OAT in response to estrogen, or in response to any other chemical stimulus.

The Examiner states that one of ordinary skill in the art would be motivated to examine both those genes, but does not explain why these particular two genes in liver cells might be selected. The Examiner has failed to provide a motivation to combine these particular two genes out of all the possible genes to measure in one assay. Indeed, Fagan *et al.*, based on the study of Mueckler *et al.*, teaches away by suggesting that the expression of OAT in the liver may not fluctuate in response to a chemical stimulus.

The Examiner appears to have used the application and the genes selected for measurement in the claims to identify two articles, each measuring expression levels for one of the genes selected in the instant invention. Numerous cases of the Federal Circuit have stated that this is not a permissible grounds for the rejection of claims.

Before the PTO may combine the disclosures of two or more prior art references in order to establish prima facie obviousness, there must be some suggestion for doing so, found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art.

In re Jones, 958 F.2d 347, 351, 21 U.S.P.Q.2d. 1941, 1943-44 (Fed. Cir. 1992).

There must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination. That knowledge can not come from the applicant's invention itself.

In re Oetiker, 977 F.2d 1443, 1447, 24 U.S.P.Q.2d. 1443, 1446 (Fed. Cir. 1992).

To establish a prima facie case of obviousness based on a combination of the content of various references, there must be some teaching, suggestion or motivation in the prior art to make the specific combination that was made by the applicant. Obviousness can not be established by hindsight combination to produce the claimed invention. As discussed in Interconnect Planning Corp. v. Feil, 774 F.2d 1132, 1143, 227 U.S.P.Q. (BNA) 543, 551 (Fed. Cir. 1985), it is the prior art itself, and not the applicant's achievement, that must establish the obviousness of the combination.

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In re Dance, 160 F.3d 1339 1343, 48 U.S.P.Q.2d. (BNA) 1635, 1637 (Fed. Cir. 1998) (citations omitted).

The claim is not directed to two or more genes from a liver originated cell. The claim is drawn to a specific set of genes to be analyzed with respect to toxin-induced differential gene expressions. Although the expression levels of both genes is measured in Diel *et al.* and Fagan *et al.*, the Examiner has provided no motivation for one skilled in the art to combine these two particular genes when measuring the response to exposure to a toxin. The Examiner is drawing together references from the literature without providing a motive to combine them. One skilled in the art would have no motivation to select these particular genes from all those identified and characterized at the time of filing other than their combination in the instant invention.

Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the objection.

The Examiner has rejected claims 1-11, 14, 15, 18 and 19 under § 103(a) as being unpatentable over Diel, *et al.* (1995) and Fagan, *et al.* (1989), and in further view of Desjardins, *et al.* (1998). Diel, *et al.* and Fagan, *et al.* have been discussed, *supra*. Applicant agrees that Desjardins used Hep2A cells to indirectly measure transcriptional responses to a chemical stimulus, a toxic material, in this case, quinone methides. However, Applicant is not clear of the relevance of this reference to the listed claims. Desjardins *et al.* are measuring the induction in expression of a non-native gene product, the chloramphenicol transferase (CAT) gene, in cells which have been transfected with a series of constructs driven by different promoters. These different promoters drive the transcription, followed by the translation, of a single open reading frame. There is no real capability of measuring the differential response in the transcription of two (or more) genes to a given chemical stimulus in the same cell at the same time.

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Additionally, the CAT gene is a bacterial gene renowned for catalyzing a chemical reaction that lacks significant background activity in mammalian cells. Therefore, Desjardins, *et al.*, are not studying the changed expression of the genes listed in claim 1 in response to toxins, nor is the CAT gene disclosed claim 1.

Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the objection.

The Examiner has rejected claim 16 under 35 U.S.C. § 103(a) as being unpatentable over Diel, *et al.* (1995) and Fagan, *et al.* (1989) in view of Schena, *et al.* (1996). Diel, *et al.* and Fagan, *et al.* have been discussed, *supra*. Schena discloses a microarray where the microarray comprises DNA sequences obtained from an expression library of a human cell. However, it is distinguishable from the instant invention as the DNA sequences present on the microarray of Schena *et al.* were selected randomly. The DNA sequences used in the instant invention are specifically set forth in claim 1. As stated earlier, one of the advantages of the instant invention is the selection of a relatively short, specific list of genes for use in assaying the effect of a toxicant by measuring the change in their expression.

Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the objection.

The Examiner has rejected claim 17 under 35 U.S.C. § 103(a) as being unpatentable over Diel, *et al.* (1995) and Fagan, *et al.* (1989) further in view of Zamorano, *et al.* (1996). Diel, *et al.* and Fagan, *et al.* have been discussed, *supra*. Zamorano *et al.* admittedly use RT-PCR to measure the expression of genes in neuroendocrine studies. In fact, Zamorano *et al.* begin their introduction (page 397) by stating "[a]n important aspect in the determination of gene expression is measuring mRNA levels of specific genes." Yet Zamorano *et al.* provide no guidance as to

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how to select any particular genes. Zamorano et al. are silent as how to select the set of genes to be used for an assay. Therefore, one of ordinary skill in the art could use the techniques described in Zamorano et al., but would be no closer in selecting which genes, out of the hundreds or thousands available, should be selected to use in measuring a toxic response. The Examiner has failed to provide a motivation to combine the particular genes set forth in claim 1 when measuring a toxic response by means of RT-PCR.

Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the objection.

The Examiner has rejected claims 28-30 under 35 U.S.C. § 103(a) as being unpatentable over Diel, et al. (1995) and Fagan, et al. (1989) further in view of Li, et al. (1989), and Martin, et al. (1996). Diel, et al. and Fagan, et al. have been discussed, *supra*. As in Desjardins, et al., above Li, et al. used reporter genes. Li et al. are measuring the induction in expression of a reporter gene, the chloramphenicol transferase (CAT) gene, in cells which have been temporarily transfected with a CAT construct driven by LDH-A promoter. Martin et al. teach the independent and combined (Dual-Light) detection method for the detection of gene expression.

Applicant wishes to point out that the recent scientific literature is and was replete with studies where cells were stimulated with a variety of stimuli in order to measure the changes in the level of gene expression. Applicant respectfully argues that the Examiner has failed to provide any motivation for one skilled in the art to combine the four references and simultaneously measure the response of three or more particular genes selected from the list in claim 28 in response to a toxin, or in response to any other toxic stimulus.

The Examiner states that one of ordinary skill in the art would be motivated to examine the regulation in gene expression of three or more different genes such as IGFBP-1, OAT, and

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lactate dehydrogenase A in response to estrogen. However, the Examiner has failed to provide a motivation to combine the particular genes listed supra or listed in claim 1 out of the hundreds or thousands of available genes, nor do the above references suggest using such a means to measure gene expression for use in screening for possible toxicants. The Examiner appears to have used the application and the genes selected for measurement in the claims to identify the articles cited for this rejection. Numerous cases of the Federal Circuit, as referenced *supra*, have stated that this is not a permissible grounds for the rejection of claims.

Therefore, Applicant respectfully requests that the Examiner reconsider and withdraw the objection.

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Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

CONCLUSION

In view of the discussion above, the Applicants submit that the claims are in condition for allowance and respectfully request a notice to that effect. Should the Examiner have any further concerns or issues, he is encouraged to contact the undersigned.

Respectfully submitted,

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Dated: April 29, 2002

By: 

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